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Research Article

Identification of molecular marker linked to mungbean yellow mosaic virus (MYMV) resistance in *Vigna radiata* (L.) Wilczek

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Abstract
Mungbean [*Vigna radiata* (L.) Wilczek], a vital source of easily digestible protein has wide adaptability and suitability to various cropping systems in India. However, it's productivity is greatly affected by mungbean yellow mosaic virus **(**MYMV) which may cause up to 100 percent yield loss. The present investigation was undertaken to study the mode of inheritance of MYMV resistance in an interspecific cross of mungbean and identify SSR marker(s) linked to it through bulked segregant analysis. MYMV susceptible *Vigna radiata* genotype SML668 was crossed as female to MYMV resistant *V. mungo* genotype Mash114 to generate F_2 mapping population. The segregation of MYMV in F_2 population fitted well in genetic ratio of 3 resistant: 1 susceptible indicating that MYMV resistance from Mash 114 was governed by single dominant gene. Bulked segregant analysis (BSA) with 67 SSR markers selected from related *Vigna* species identified 46 markers polymorphic between the parental lines while one SSR marker- MBM 0378 amplified a polymorphic fragment between resistant bulk and susceptible bulk indicating association of this marker with MYMV resistance.

Key words
V. radiata, *V. mungo*, MYMV, SSR and bulked segregant analysis

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] is an important short duration widely cultivated grain legume crop of India. It has three times more protein content than cereals, high amino acid content of lysine and tryptophan, low proportions of flatulence factors (Gosal and Bajaj, 1983) and iron concentrations of 40-70 ppm (Weinberger, 2005) making it an attractive crop for balanced diets in cereal-based vegetarian food systems in South and South-east Asia Further its nitrogen-fixing ability improve the soil health by improving soil fertility and texture (Graham and Vance, 2003).

Among the various diseases that affect mungbean productivity, mungbean yellow mosaic virus (MYMV) , which is a Gemini virus is most prevalent in South Asian countries, viz. India, Sri Lanka, Pakistan, Bangladesh and in severe form causes significant grain yield losses. It is transmitted by whitefly, *Bemisia tabaci* (Nene, 1972) which can be controlled by timely application of insecticides. But the cost and non-availability of pesticides hinders the effective control of the disease. Also, under severe whitefly infestation the use of insecticides is ineffective. Moreover, it is also not an eco-friendly approach. So, the most effective way to combat this disease of mungbean is to introduce long term resistance from related species like urdbean [*Vigna mungo*(L.) Hepper], through interspecific hybridization. Urdbean, *V. mungo* (L.) Hepper possesses resistance to various foliar diseases which can be transferred to mungbean through hybridization (Singh 1990). One of the urdbean variety Mash 114, used in the present study is highly resistant to MYMV as it has introgression of desirable traits from ricebean (Singh *et al.,* 2013) There are several reports of attempting interspecific crosses between mungbean and urdbean in order to transfer resistance to various foliar diseases in mungbean (Abbas *et al.,* 2015 and Sherawat *et al.,* 2016). In these studies molecular markers have been used to confirm the putative F_1 hybrids.

Few ISSR based SCAR, RAPDs, RGA and SSR markers are found to be linked with MYMV resistance in urdbean and mungbean (Selvi *et al.,* 2006; Souframanien and Gopalkrishnan, 2006; Karthikeyan *et al.,* 2012 and Gupta *et al.,* 2013). However, due to their limited application in marker assisted breeding, immediate efforts are required to develop MAS-worthy, MYMV resistance linked markers. Recently, microsatellite markers have turned out to be most important tool for marker

assisted breeding in several crops as they are highly polymorphic, easy to use for screening large segregating populations and reproducible. In earlier studies, development of SSR markers has been reported in many *Vigna* species and other pulse crops (Kumar *et al.,* 2011). Many studies on disease resistance genes have shown a high level of polymorphism because of the presence of SSR at certain loci (Yu *et al.,* 1996).Therefore, the present investigation was carried out with an aim to identify some SSR markers linked to MYMV resistance gene(s) in mapping population developed from interspecific cross.

Material and Methods

The experimental material consisted of 150 F_2 plants developed from the cross SML668 with Mash 114. SML668 is a high yielding variety of summer mungbean cultivated in Punjab during spring/summer season and is highly susceptible to MYMV. Mash 114 is a high yielding variety of urdbean cultivated in Punjab during *kharif* season and is highly resistant to MYMV and other foliar diseases. SML668 was crossed as female with Mash114 during summer 2015 and an F_2 mapping population comprising of 150 plants was developed.

The infector row method was used for disease evaluation in which two test rows alternating with spreader rows of highly susceptible variety SML 1082 were sown at PAU Ludhiana which is a hot spot for MYMV. The F_1 plants and each plant of F_2 mapping population was scored for disease incidence during *Kharif* 2016. All recommended cultural practices were followed, except spraying of insecticide. Disease reaction was scored twice at an interval of one week, when infector rows showed full MYMV incidence under field conditions. The disease was scored on 0-9 scale (Singh *et al.,* 1992) with plants having score of 0-4 were considered as resistant while plants with MYMV score more than 4 were considered as susceptible (Table 1). Numbers of resistant and susceptible F_2 plants were calculated to determine the number of gene(s) controlling the MYMV resistance. Chi-square test was used to check goodness of fit for ascertaining the number of genes governing the MYMV resistance

Total DNA was extracted from each of F_2 plant and parental lines following CTAB method (Doyle and Doyle, 1987). The resistant bulk (RB) and susceptible bulk (SB) were prepared by mixing equal volume of DNA from 10 resistant (MYMV Score < 4) and 10 susceptible plants (MYMV Score > 4) of F_2 Population. Bulked segregant analysis (BSA) was done on parental lines, RB and SB using polymorphic SSR markers. A set of 67 SSR primers were selected for present study which includes 22 SSRs (MBM) were derived from EST sequences of mungbean (Gupta *et al* 2014), 31 SSRs (VR) were from shot gun sequences of mungbean (Tangphatsornruang *et al* 2009), while 14 SSRs (Ced) were from genomic sequences of azuki bean (Wang *et al* 2004). SSR markers displaying differential amplification pattern between resistant and susceptible bulks were identified as markers co-segregating with MYMV.

PCR reactions were performed in 10 µl volume containing 2.0 µl genomic DNA, 1.5 µl dNTPs, 2.0 µl PCR buffer (5X), 0.8 µl $MgCl_2$, 0.8 µl of both forward and reverse primers, 1.0 µl Taq polymerase and 1.1 µl autoclaved distilled water. PCR amplifications were performed in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) using the following thermal profile: 1 cycle of 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min, annealing for 1 min at respective temperature of primer, 72 °C for 1 min and a final extension of 72 °C for 10 min. PCR product was resolved in six percent polyacrylamide gel (PAGE) prepared in 0.5X TBE buffer was used for resolution of amplified DNA fragments.

Results and Discussion

Mungbean yellow mosaic virus disease (MYMV) is a major problem in production of mungbean leading to high grain yield losses from 10-100 percent. It is caused by mungbean yellow mosaic India virus (MYMIV) in northern and central India. Whereas, disease in southern and western India is caused by mungbean yellow mosaic virus (MYMV) (Usharani *et al.,* 2004).

The data on reaction to mungbean yellow mosaic virus (MYMV) disease of parental lines SML668, Mash114 their F_1s and F_2 plants is given in Table 2. SML 668 was susceptible with MYMV score 9 whereas Mash 114 was resistant with MYMV score 1. All the F_1 plants from SML 668 x Mash 114 were resistant indicating the dominant nature of genes contributing towards resistance. Out of 150 F_2 plants, 111 were resistant with MYMV score 0-4 while 39 plants with MYMV reaction 5-9 were scored as susceptible. This segregation of resistant and susceptible F_2 plants fits into 3:1 ratio ($\chi 2$= 0.08), thereby confirming to monogenic inheritance of MYMV resistance from Mash 114 which is dominant in nature. Of the 67 SSR markers used in present study 46 were found to be polymorphic between the parental lines SML 668 and Mash 114 and were further used to amplify resistant bulk (RB) and susceptible bulk (SB). Out of 46 polymorphic

primers, only one SSR marker namely MBM 0378 amplified PCR product of 135 bp in resistant bulk, similar to Mash 114 and amplified PCR product of 150 bp present in susceptible bulk similar to susceptible parent SML 668 (Fig.2). Rest polymorphic markers were not able to distinguish the resistant and susceptible bulks.

Similar inheritance studies of MYMV have been carried out earlier in which resistance is reported to be governed by one or two genes (monogenic or digenic inheritance) with different type of gene interaction. Inheritance of MYMIV resistance gene was studied in black gram using F_1, F_2 and $F_{2:3}$ produced from cross DPU 88-31 (resistant) × AKU 9904 (susceptible). The results of genetic analysis depicted that a dominant gene controls the MYMIV resistance in black gram genotype DPU 88-31 (Gupta *et al* 2013). Similarly, Kumari *et al* (2015) studied genetics of resistance to MYMV in black gram by using F_2 and F_3 populations produced from a cross between the susceptible parent TAU-1 and the resistant parent BDU-4 under artificial conditions. The results depicted that the yellow mosaic disease resistance in black gram is controlled by a dominant gene. However monogenic recessive control of yellow mosaic disease resistance was observed in intraspecific crosses of mungbean by Jain *et al* (2013) and Sudha *et al* (2013).

To identify SSR markers closely associated with MYMV resistance gene BSA was done.

BSA involves screening of contrasting bulks made from individuals in a segregating population (Michelmore *et al.*, 1991) reducing time consuming procedure of screening entire population with every primer. Souframanien and Gopalkrishna (2006) reported a SCAR marker linked to MYMV resistance gene with linkage distance of 6.8 cm, but the virus species causing yellow mosaic disease (YMD) was not clearly identified. Maiti *et al* (2011) also identified molecular markers linked to MYMV resistance which could be used for genotyping of urdbean and mungbean germplasm.

Gupta *et al.* (2013) used BSA to identify an SSR marker; CEDG 180 linked with yellow mosaic resistance gene in blackgram in an F_2 population derived from cross DPU 88-31 (resistant) and AKU 9904 (susceptible). However the marker CEDG 180 did not show any linkage to MYMV resistance in the present study. Karthikeyan *et al* (2012) found RAPD marker OPB 05 linked to mungbean yellow mosaic disease resistance gene in mungbean. Holeyachi and Savithramma (2013) also identified random amplified polymorphic DNA (RAPD) marker UBC 499 associated with yellow mosaic resistance in mungbean (*Vigna radiata* (L). Wilczek) through bulked segregant analysis.

Marker assisted selection (MAS) has nowadays become an essential tool in breeding programs for those traits which are difficult to screen phenotypically. Molecular markers are now extensively used to track loci and genomic regions in various legume crops and a large number of improved varieties have been developed using these markers in recent times (Kumar *et al* 2011). Moreover field screening of MYMV is also difficult since it requires evaluation at 'hot spot' area. Being seasonal it is difficult to create disease as and when required by artificial means.

Many commercial varieties of mungbean such as SML 668; have now become susceptible to MYMV, so there is need to identify tightly linked markers that could be helpful in transferring resistance genes to such popular cultivars through marker assisted breeding. Moreover, molecular markers linked to resistance genes are useful since they remove the delays in breeding programs associated with the phenotypic analysis. Thus, SSR marker, MBM 0378 identified as associated with MYMV resistance in the present study will be useful in the identification of resistance sources which will further enhance the accuracy in MYMV resistance breeding program and could be used in future for the development of high yielding mungbean yellow mosaic disease resistant cultivars of mungbean .

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Table 1. Rating Scale for MYMV (Mungbean Yellow Mosaic Virus) disease incidence as per Singh *et al.*(1992)

Rating	Percentage Foliage Affected	Reaction
1	No phenotypic symptoms or small yellow specks covering about 0.1-5 percent leaf area	Highly resistant
3	Mottling of leaves covering about 5.1-15 percent leaf area	Resistant
5	Yellow discoloration and mottling of 15.1-30 percent leaf area	Moderately resistant
7	Noticeable yellow mottling and discoloration of pods, leaves, reduction in leaf size, stunting of plants, 30.1-75 percentage foliage affected	Susceptible
9	Intensive yellow mottling and discoloration of leaves , failure of flowering , stunting of plants and seed setting, 75.1-100 percent foliage affected	Highly susceptible

Table 2. MYMV disease reaction of F_1 and F_2 generation of an interspecific cross of mungbean under natural field conditions

Generation		Resistant Plants	Susceptible Plants	Total Plants	Expected ratio	$\Sigma\chi^2$	Probability
F_1		5	0	5	-	-	-
F_2	Observed	111	39	150	3:1	0.08	0.78
	Expected	113	37	150	-	-	-

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Fig.1. Leaves of Parents and F_1 hybrid showing mungbean yellow mosaic disease reaction.

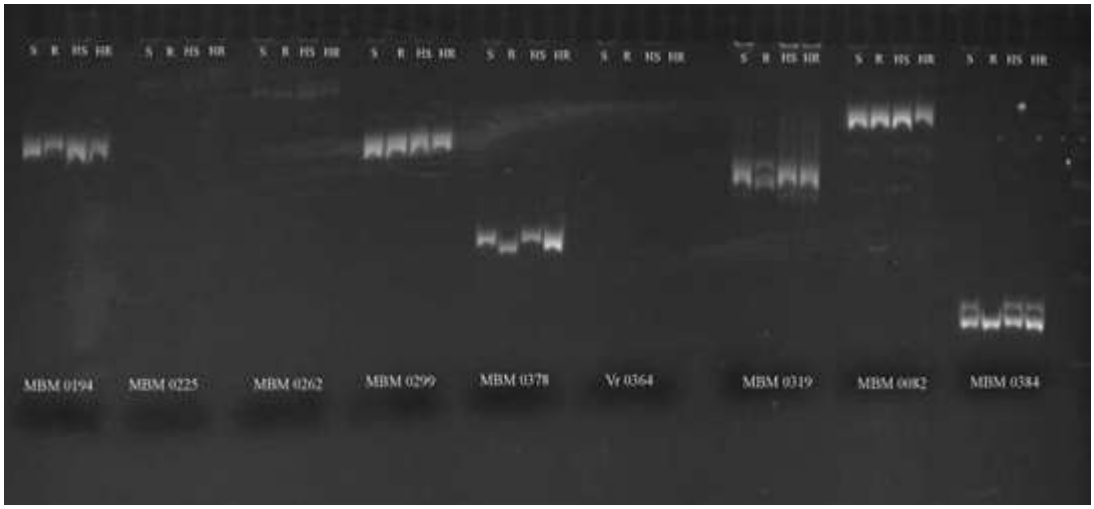

Fig. 2. PCR amplification profile of Parental lines(S and R), Susceptible Bulk(HS) and Resistant Bulk (HR)
Names written on lowermost end of slides represent name of microsatellite used .
S: Susceptible, R: Resistant; HS : Highly Susceptible , HR: Highly Resistant.